Repeated climate-linked host shifts have promoted diversification in a temperate clade of leaf-mining flies

Isaac S. Winkler[a,b,1], Charles Mitter[b], and Sonja J. Scheffer[c]

[a]Department of Entomology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613; [b]Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park, MD 20742; and [c]Systematic Entomology Laboratory, Plant Science Institute, Agricultural Research Service, United States Department of Agriculture, 10300 Baltimore Avenue, Building 003, Room 231, BARC-West, Beltsville, MD 20705

Edited by Anurag A. Agrawal, Cornell University, Ithaca, NY, and accepted by the Editorial Board July 30, 2009 (received for review May 1, 2009)

A central but little-tested prediction of "escape and radiation" coevolution is that colonization of novel, chemically defended host plant clades accelerates insect herbivore diversification. That theory, in turn, exemplifies one side of a broader debate about the relative influence on clade dynamics of intrinsic (biotic) vs. extrinsic (physical-environmental) forces. Here, we use a fossil-calibrated molecular chronogram to compare the effects of a major biotic factor (repeated shift to a chemically divergent host plant clade) and a major abiotic factor (global climate change) on the macroevolutionary dynamics of a large Cenozoic radiation of phytophagous insects, the leaf-mining fly genus *Phytomyza* (Diptera: Agromyzidae). We find one of the first statistically supported examples of consistently elevated net diversification accompanying shift to new plant clades. In contrast, we detect no significant direct effect on diversification of major global climate events in the early and late Oligocene. The broader paleoclimatic context strongly suggests, however, that climate change has at times had a strong indirect influence through its effect on the biotic environment. Repeated rapid Miocene radiation of these flies on temperate herbaceous asterids closely corresponds to the dramatic, climate-driven expansion of seasonal, open habitats.

adaptive radiation | coevolution | climate change | macroevolution | Agromyzidae

Students of macroevolution have long debated the relative importance of intrinsic traits, such as those governing biotic interactions or genetic population structure, versus extrinsic physical factors, such as climate change or tectonic events, in determining rates of lineage proliferation and extinction (1, 2). The intrinsic view is exemplified by the neodarwinian postulate that episodes of diversification result from the evolution of "key innovations" opening access to previously unavailable resources (3). Strong examples support the operation of intrinsic and extrinsic forces in isolation (4). Only recently, however, has the debate moved toward synthesis, i.e., joint estimation of the relative importance of each, and their interaction, at different time scales (2, 4, 5). In this study we compare the effects of a major intrinsic factor (shift to a novel host plant clade) and a major extrinsic factor (global climate change) on the macroevolutionary dynamics of a temperate, Cenozoic radiation of phytophagous insects, the leaf-mining fly genus *Phytomyza* (Diptera: Agromyzidae).

Insect herbivores became a model for biotic influences on macroevolution through Ehrlich and Raven's (6) influential "escape and radiation" model of coevolution, under which the origins of novel plant defenses, and of corresponding insect counterdefenses, constitute key innovations promoting the diversification of lineages that bear them. There is now extensive support for some aspects of Ehrlich and Raven's hypothesis, most notably the dominant influence of plant secondary chemistry on the evolution of insect host plant use (7, 8). Despite a recent surge of interest in insect diversification rates (9), however, there is still little evidence on the degree to which changes in either plant defense or insect "offense" promote diversification (7). Progress on the insect side has come from several recent reports plausibly attributing an instance of significantly elevated insect diversity to a co-occurring shift to a new host taxon (5, 10, 11). Any single instance of elevated diversification, however, could reflect other influences that happen to be confounded with the host shift. The causal link to host use would be stronger if multiple instances of analogous host shifts could be identified and shown to be consistently associated with higher diversification. The only such repeated association thus far reported is elevated diversity of angiosperm vs. gymnosperm feeders, among major clades of Phytophaga beetles (12, 13) and in basal Lepidoptera (14).

Phytomyza is an apt group for pursuing this question because it features repeated shifts from a relatively ancient angiosperm clade, ranunculids, to a much younger and chemically very different host clade, the asterids. *Phytomyza*, with >700 described species (15), includes approximately a quarter of total diversity in the family Agromyzidae, larvae of which form characteristic mines (Fig. 1) in leaves or other tissues of many different plant families (16, 17). The genus is chiefly north temperate, with >90% of species occurring only in the Nearctic or Palearctic bioregions (15). It has long been considered to consist in major part of distinct species groups, each containing species that are morphologically homogeneous and feeding primarily on plants in the same family or order (16). Our recent molecular-phylogenetic study (15) sampled 108 *Phytomyza* species, representing nearly all species groups, for 3,076 bp of sequence from one mitochondrial and two nuclear gene regions. The resulting tree strongly corroborated the general validity of the species group concept, while clarifying the composition of and relationships among species groups. Morphological evidence was then used to place nonsequenced species on the tree, yielding the clade diversity estimates shown in Fig. 2. This foundation now enables us to use an exemplar-based approach for studying diversification at an otherwise problematic taxonomic scale.

We also take advantage of the recent discovery of fossilized leaf mines that extend the confirmed fossil history of Agromyzidae by ~15 Ma, to the earliest Paleocene (18). Upon further study of these fossils (Fig. 1 *D* and *E*) we noted three features that

Author contributions: I.S.W., C.M., and S.J.S. designed research; I.S.W. and S.J.S. performed research; I.S.W. analyzed data; and I.S.W. and C.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. A.A.A. is a guest editor invited by the Editorial Board.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. EU367919–EU367931).

[1]To whom correspondence should be addressed. E-mail: iswinkle@ncsu.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0904852106/DCSupplemental.

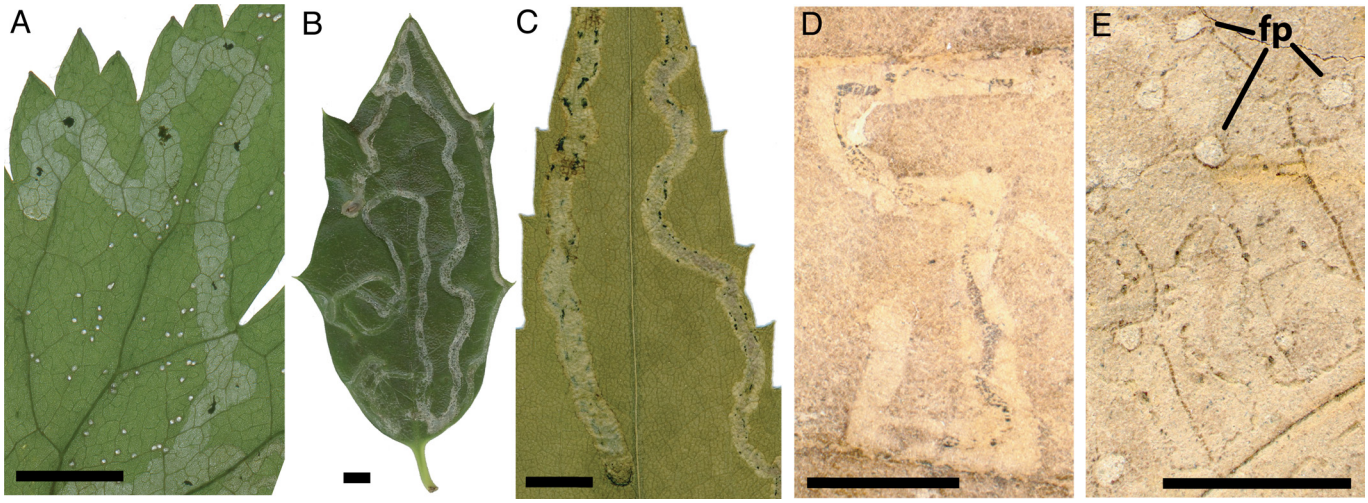

Fig. 1. Examples of extant *Phytomyza* (*A*–*C*) and fossil (*D* and *E*) leaf mines. (*A*) Mine of *Phytomyza* sp. on *Trautvetteria caroliniensis* (Ranunculaceae; Idaho) showing feeding punctures diagnostic of agromyzid damage. (*B*) Mine of *Phytomyza opacae* on *Ilex opaca* (Aquifoliaceae; Maryland). (*C*) Mine of *Phytomyza solidaginophaga* on *Solidago canadensis* (Asteraceae; Colorado). (*D* and *E*) Fossil leaf mines on *Platanus raynoldsi* (Platanaceae, Paleocene of Montana; see ref. 18), showing feeding punctures (fp). (Scale bar, 5 mm.)

securely diagnose them as agromyzid mines: an intermittent fluidized frass trail, alternation of frass bands between the two sides of the mine during at least some stages, and a series of small holes near some of the mines (Fig. 1*E*). The latter are identical to the unique punctures made by extant agromyzid females with their ovipositors before egg-laying (Fig. 1*A*). Feeding from these punctures is thought to guide host plant recognition, and promotes female survival and fecundity (19, 20). These and other fossils, combined with a recent molecular phylogeny that sampled broadly across the family (17), allow us to estimate minimum ages of agromyzid genera, including *Phytomyza*. Those ages, in turn, provide calibration for detailed divergence time estimation within *Phytomyza*, of which there are no confirmed pre-Pliocene fossils.

Phytomyza species, each typically restricted to one or a few closely related species of herbs, feed mostly on plant families that are diverse and abundant in temperate regions (15, 16). The main hosts fall into two disparate groups: Ranunculaceae, belonging to the oldest clade of eudicots (ranunculids); and asterids, especially Asteraceae, Apiaceae, Lamiaceae, Orobanchaceae, and Plantaginaceae, the last three belonging to the order Lamiales (21). Both of these clades possess conspicuous, presumably defensive secondary chemistry. Ranunculaceae variously produce several alkaloids or the glycoside ranunculin (22), while the asterid orders and families collectively produce an astounding variety of characteristic compounds. For example, iridoid glycosides are prominent in Lamiales, seco-iridoids in Dipsacales, sesquiterpene lactones in Asteraceae, and coumarins and acetylenes in Apiales (23).

Spencer (16), noting that *Phytomyza* species on Ranunculaceae are morphologically exceptionally heterogeneous, postulated an original association with Ranunculaceae, followed by later shifts to other host families, mostly asterids. We initially hypothesized that shifts from Ranunculaceae to asterid hosts were contemporaneous with the earliest diversification of north temperate asterid lineages. Codiversification in this sense now seems very unlikely, as the earliest lineages of *Phytomyza* proved not to feed on Ranunculaceae (15), and many asterid herb lineages probably date to the early Cenozoic or earlier (24). Nonetheless, Ranunculaceae feeding does appear to have evolved early in the history of the genus, and given rise repeatedly to asterid feeding. We have re-examined the history of host shifts in *Phytomyza*, identifying multiple comparisons for diversification rates in Ranunculaceae feeders vs. asterid feeders derived from these (Figs. 2 and 3). We then tested the prediction that diversification is consistently elevated by shifts to a novel host clade (asterids) of high species and secondary-chemical diversity.

Changes in biotic interactions, such as host plant use, may often be triggered by geographic shifts or climatic changes that alter the biotic environment (7, 25). Here, we have exploited the relatively well-studied history of the boreo-temperate region since the middle Eocene to place the *Phytomyza* radiation in paleoclimatic and geographic context. That history, summarized in Fig. 4, includes a strong overall trend toward cooling, drying, seasonality, and latitudinal climate stratification (26–28), punctuated by the rapid cooling events of the earliest Oligocene glacial maximum (EOGM, 33.5 Ma) (29) and the middle Miocene climatic transition (MMCT, 13.9 Ma) (30). The cooling trend was interrupted from the late Oligocene warming event (LOWE, 24–26 Ma) until the middle Miocene (28). Overall, however, plant communities in the middle latitudes of the northern hemisphere shifted from warm-adapted toward cool-adapted modes (26, 31, 32), with pronounced expansion of open habitats and herbaceous vegetation including grasses and composites after the MMCT. The Miocene event in particular probably spurred diversification of many temperate herb lineages (33), and possibly radiations of associated insect herbivores as well (34). Against this backdrop, we used the time-calibrated phylogeny to determine whether major climate transitions are coincident with shifts between major host clades in *Phytomyza*, and to assess the relative effects of climate vs. host change, and their interaction, on diversification dynamics.

Results

Divergence Times. The group names used here follow ref. 15 (see Fig. 2). Divergence time estimation using the all-genera dataset (including 33 *Phytomyza*; Fig. S1), with three agromyzid fossils as calibration, returned a stem group (root) age for *Phytomyza* of 44.0 Ma (Bayesian: 42.7 ± 5 Ma) and crown group age of 39.1 Ma. The crown-group age of the *Phytomyza s.str.* clade was estimated at 32.8 Ma (Bayesian: 32.1 ± 4 Ma). Using these ages to calibrate the densely sampled phylogeny of *Phytomyza* (Fig. 2) yielded an estimated age for the major radiation ("*Phytomyza* main lineage," or PML clade) of ~24.5 Ma with subsequent

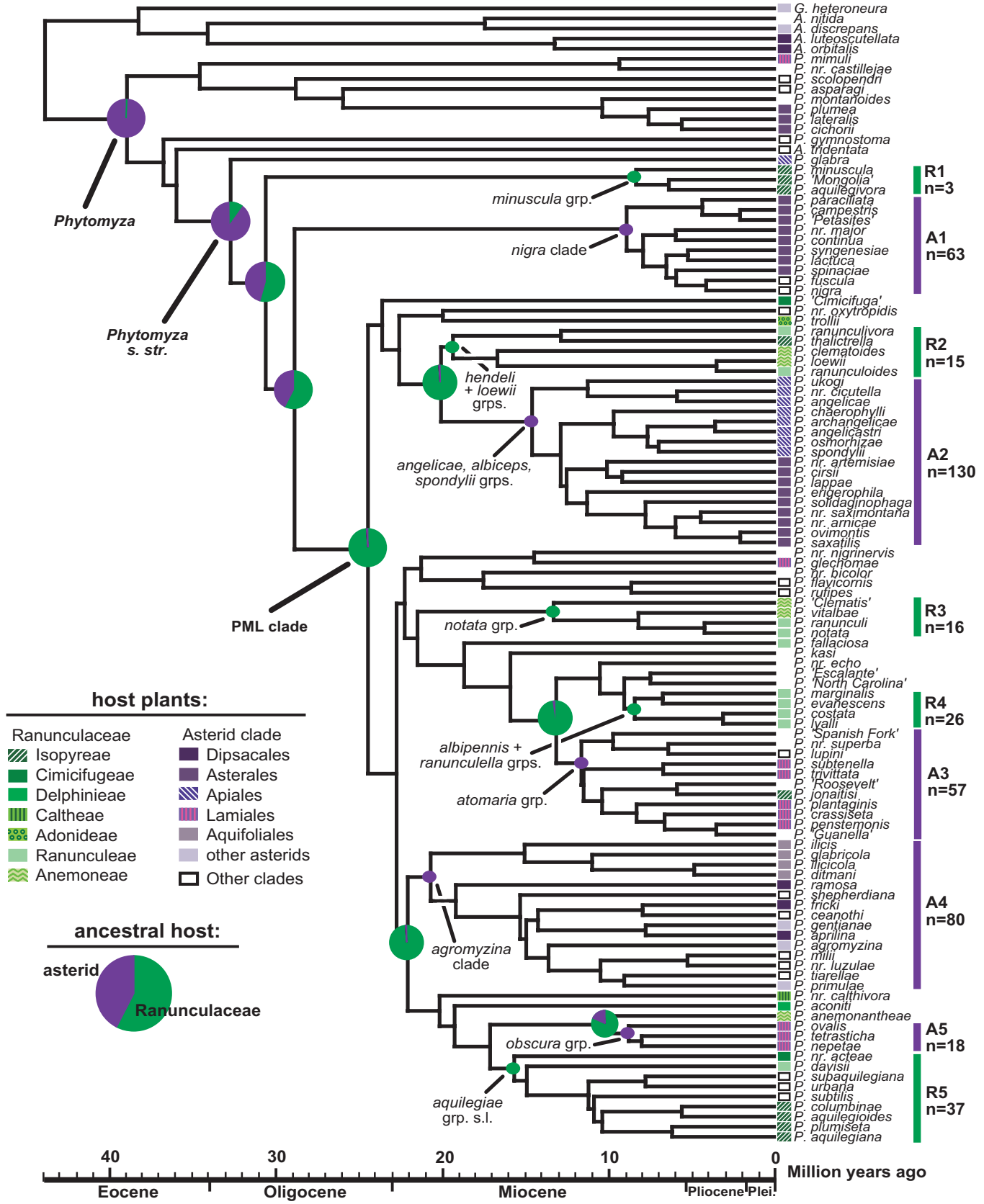

Fig. 2. Phylogeny of *Phytomyza*, with divergence times estimated by the PL method. The stem group age of *Phytomyza* (44.0 Ma) and the crown group age of *Phytomyza s.str.* (32.8 Ma) were fixed according to the results of fossil-calibrated divergence time estimation of the family-wide data. Host plants (tribes of Ranunculeae, orders of asterids) are indicated by colored boxes at right, and proportional likelihoods of ancestral host clades (purple, asterids; green, Ranunculaceae) for selected nodes are shown in pie diagrams (proportions for other host clades are negligible). Clades used to test host-related diversification rate variation are marked at right (R1–R5, A1–A5). Topology and clade names are from ref. 15.

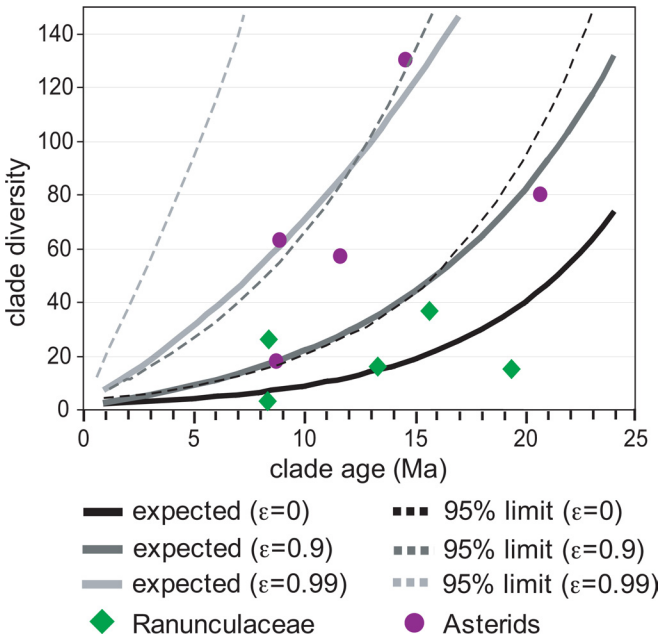

Fig. 3. Diversity vs. clade age for 10 *Phytomyza* clades feeding on asterid (purple circle) or ranunculaceous (green diamond) hosts. Expected clade sizes according to a constant-rate birth-death model are shown by black (no extinction) and gray (ε = 0.9/0.99) lines, along with confidence intervals. Under the assumption of no extinction, four asterid-feeding clades are significantly more diverse than expected, while only one Ranunculaceae-feeding clade is such.

radiations of species groups beginning mostly between 14 Ma and 8 Ma.

Ancestral Hosts. Maximum likelihood (ML) reconstruction assigned an asterid host to the ancestor of *Phytomyza s.lat.* and *Phytomyza s.str.* [relative likelihoods (RL) = 0.98 and 0.89, respectively], but strongly favored Ranunculaceae as the ancestral host for the PML clade (RL = 0.99). Bayesian reconstructions for the latter two nodes were similar but less conclusive (posterior probability = 0.74 for *Phytomyza s.str.*, 0.86 for the PML clade). Relative likelihoods for intermediate nodes were equivocal, suggesting a shift to Ranunculaceae some time between the origin of these two clades (32.8–24.5 Ma). An early shift to Ranunculaceae was slightly favored (RL = 0.57), implying, although not definitively, that asterid-feeding in the *nigra* clade is secondary. Within the PML clade, ML reconstructions strongly favor separate origins of asterid feeding from Ranunculaceae-feeding ancestors (Fig. 2). Relative likelihoods for feeding on other plant clades were negligible at each of these nodes.

Diversification Rates and Host Shifts. Under a simple birth-death model (35), the overall diversification rate for *Phytomyza* was estimated as 0.15 per Ma assuming zero relative extinction (ε = 0), and 0.11/Ma and 0.05/Ma assuming ε = 0.9 and ε = 0.99, respectively. On average, asterid-feeding clades had higher rates of diversification than Ranunculaceae-feeding clades (0.278/Ma vs. 0.160/Ma for ε = 0; Fig. 3 and Table S1). These differences were significant under both likelihood ratio tests (one-tailed; P = 0.004/0.022/0.007 for ε = 0/0.9/0.99; see Table S2) and the Mann–Whitney U test (one-tailed; P = 0.02 for all values of ε). The results were essentially unchanged when the diversities of primarily asterid- or Ranunculaceae-feeding clades were adjusted by excluding member species that feed on other plant clades (P = 0.03).

Diversification Rates and Climate Change. Under two ML-based methods fitting pure birth (Yule) models to branching times (36, 37) diversification rates were inferred to be substantially higher before the EOGM cooling event than after, and to increase again after the LOWE warming event (Fig. 4 and Table S3). For only one of these comparisons, however, did the model incorporating rate shifts show a better fit under the Akaike information criterion (AIC) than a constant rate model, and none were significant under a likelihood ratio test. Parametric simulation of phylogenies to explore the effects of incomplete lineage sampling on the ML test confirmed that the observed rate differences were within the limits of expected variation under the single-rate Yule model for a range of numbers of missing lineages (see Table S3).

Discussion

History of Major Host Shifts. According to our analysis, the earliest members of crown group *Phytomyza* (middle Eocene, 39.1 Ma)

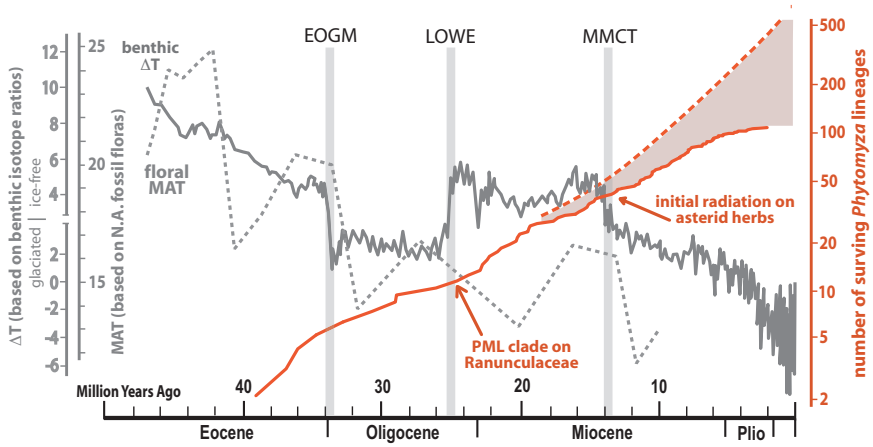

Fig. 4. LTT plot of *Phytomyza* species accumulation over time (red) superimposed on temperatures estimated by isotopic ratios of benthic foraminifera (ref. 28; solid gray line, modified from www.globalwarmingart.com) and leaf analysis of North American fossil floras (ref. 27; dotted gray line). Temperature variation based on isotope ratios is not as dramatic as it appears because the calibration differs between glaciated and unglaciated states (see scale bars at left). Lineage sampling is assumed to be approximately complete before 19 Ma; the dotted red line represents a possible subsequent trajectory of the LTT curve with the addition of unsampled *Phytomyza* species. Major climatic events are indicated by gray bars as follows: EOGM, early Oligocene glacial maximum; LOWE, late Oligocene warming event; MMCT, middle Miocene climatic transition. MAT, mean annual temperature.

almost certainly fed on asterids (Fig. 2). In the late Eocene or Oligocene, a lineage giving rise to most extant species subsequently shifted to Ranunculaceae, either once in the ancestor of *Phytomyza s.str.* exclusive of *Phytomyza glabra* (slightly favored by relative likelihoods), or twice somewhat later, most probably in the ancestors of the *minuscula* group and the PML clade. Radiation on the new hosts was especially extensive in the PML clade: species groups plus additional unplaced species feeding on Ranunculaceae are scattered widely through the clade, often specializing on different, chemically divergent host tribes (22). Multiple lineages within the PML clade later shifted back to asterids, mostly during the Middle Miocene.

Host Shifts and Diversification. The significant rate difference we observed between sets of five independent clades each of ranunculid and asterid feeders provides strong evidence that subsequent colonization of asterids was consistently accompanied by elevated net diversification. Indeed, the three largest asterid-feeding clades together contain more than one-third of all *Phytomyza* species, and have an average diversification rate twice that of the genus as a whole. This is only the second statistically defended example of a consistent trend toward elevated net diversification upon shift to a chemically distinctive new host plant clade, a fundamental prediction of escape and radiation coevolution (6). It is the first such finding at a low (infrageneric) insect-taxonomic level. Together with recent reports of elevated diversification linked to single host shifts within insect genera or families (5, 10, 11), it suggests that host-plant-related effects on phytophagous insect diversification may be accessible to phylogenetic study at many levels.

While our finding is consistent with chemically mediated escape and radiation coevolution sensu Ehrlich and Raven (6), enhanced radiation on asterid herbs could have been driven by several alternative mechanisms. Marvaldi et al. (13) suggested that access to vastly more diverse resources, rather than accelerated diversification prompted by colonization of a novel host clade per se, may account for the greater diversity of angiosperm vs. gymnosperm feeders among weevils. The 10 *Phytomyza* clades, like many other groups (38, 39), show little evidence for the positive correlation of diversity with age expected under simple diversification models, while the significantly elevated diversity of asterid feeders holds even when clade ages (closely matched between the two host groups) are ignored (see *SI Methods*). Thus, higher equilibrium "carrying capacity" (39) for phytophage diversity on taxonomically and chemically diverse host plant clades is a plausible alternative explanation (not mutually exclusive) to the accelerated diversification predicted by escape and radiation coevolution. Whatever the cause, a general relationship between host clade diversity and insect diversification, if upheld for other groups, would be a valuable benchmark in the search for phylogenetic influences on phytophagous insect diversity and community structure.

Climate Change and Diversification. Contrary to our findings on host shifts, we can see no clear evidence of a direct effect of major Oligocene climate events on *Phytomyza* diversification. Despite much recent interest in such questions (5, 40, 41), there is still very little quantitative evidence on the degree to which global climate events have affected insect diversification.

Our sampling design precludes a direct test for an effect of Miocene climate changes on *Phytomyza* diversification. It seems likely, however, that these abiotic events had a powerful indirect influence on *Phytomyza* clade dynamics, through their effect on host plant evolution and community composition (see also ref. 11). *Phytomyza* probably originated as a temperate-adapted clade during the gradual cooling of the late Eocene, before cool temperate biomes became widespread in the northern hemisphere. The early radiation on Ranunculaceae followed dramatic post-EOGM expansion (31, 32) of the cool deciduous woodland habitats still favored by that plant family today (42). All of the current asterid host families were probably present long before colonization by *Phytomyza* (21, 32). However, the relative diversities and abundances of those families in the north temperate zone were at first very different from today (32, 33). Herbaceous asterid groups increased dramatically in abundance and diversity in north temperate regions as cool, open habitats expanded immediately after the middle Miocene climatic transition (MMCT, 13.9 Ma). The main radiations of asterid-feeding *Phytomyza* followed almost immediately. For example, pollen of Asteraceae is present in some early Oligocene deposits in North America, but becomes abundant only following the MMCT (43); very soon after, this family was colonized by the ancestor of the species-rich *albiceps* group.

Conclusion

Benton (2) argued that biotic interactions ("Red Queen effects") primarily affect evolution at short time scales, while tectonic and climatic processes dominate at larger scales ($>10^5$ years). Our findings, in contrast, suggest that in *Phytomyza*, biotic interactions have strongly influenced diversification patterns over millions of years, with climate change contributing indirectly through its effect on the biotic environment. Indeed, our observations can readily be expressed in the imagery of the Modern Synthesis, in which diversification is a response to ecological opportunity provided by key innovations, extinction, or creation of new habitats. Already adapted to cold temperate climates and well-defended herbaceous plants, *Phytomyza* species were able to quickly exploit, through repeated host shifts, the ecological opportunity presented by climate-driven Miocene expansion of asterid-dominated herbaceous vegetation. This narrative shares elements with escape and radiation coevolution—including episodes of dramatic radiation by associated insects and plants—but brings greater realism to that scenario by factoring in the critical roles of geographic and climatic history.

Methods

(see *SI Methods* for additional details on each procedure)

Datasets and Phylogeny Estimation. We based our analyses on two separately published molecular datasets that overlap partially in gene and taxon sampling. The first of these, broadly sampling lineages across Agromyzidae (17), was augmented with sequence data from 13 additional species of *Phytomyza* reported in ref. 15 or newly generated, and reanalyzed with the ML program GARLI v.0.951 (44) under default parameters. For the second dataset, restricted to *Phytomyza* and close relatives, we used the published ML tree (15).

Divergence Time Calibration. Divergence time estimation was performed on the family level tree, using penalized likelihood (PL) (r8s v.1.71) (45) and Bayesian MCMC analysis (BEAST v.1.4.5) (46). For both methods, the root node was fixed (or tightly constrained) using the age (64.4 Ma) of the agromyzid trace fossil reported in ref. 18, with two additional fossils used as minimum age constraints. PL analysis of the *Phytomyza* only phylogeny was performed using fixed constraints obtained from the family-wide PL analysis (32.8 Ma for the crown group of *Phytomyza s.str.* and 44.0 Ma for the stem group of *Phytomyza s.lat.*).

Host Shift Reconstruction. To test the hypothesis of an early shift to Ranunculaceae with subsequent shifts to asterid families, we first pruned taxa with unknown hosts from the ML phylogeny of ref. 15. Each remaining species was scored as feeding on one of six high-level plant clades (21): Ranunculaceae, asterids, rosids, monocots, Saxifragales, Polypodiopsida. Proportional likelihoods of each state at each ancestral node were then calculated (Mesquite 1.06) (47) using a single-rate ML model (48). Bayesian posterior probabilities of ancestral states, accounting for uncertainty in tree topology and branch lengths, were also assessed (MrBayes 3.1.2) (49) for two nodes of special interest (*Phytomyza s.str.* and the PML clade).

Host Effects on Diversification. The most straightforward test for elevated diversification associated with repeated shift from ranunculid to asterid feeding would be sister group analysis (50), but very few relevant sister groups could be confidently identified. Instead, we contrasted estimates of absolute diversification rates (38) calculated independently for multiple clades of Ranunculaceae ($n = 5$) and asterid ($n = 5$) feeders within *Phytomyza s.str.* The latter each represent a probable independent shift from Ranunculaceae feeding (Fig. 2). We first estimated an overall rate for *Phytomyza* under a simple birth-death model (35). From this rate we generated expected clade sizes, and probabilities for the observed sizes, for each of the 10 clades under three assumptions about relative extinction rate: low ($\varepsilon = 0$), high ($\varepsilon = 0.9$), and very high ($\varepsilon = 0.99$). We tested for greater diversification in asterid feeders by using a likelihood ratio test, asking whether fit was significantly improved by estimating separate rates for ranunculid vs. asterid feeders, as opposed to a single average rate. As a nonparametric alternative, we also looked for significant host-associated differences in the ratio of observed to expected clade sizes using the Mann–Whitney *U* test. To investigate possible violations of the assumptions of the simple birth-death model and their effect on our results, we tested the correlation between age and diversity of the two sets of clades (39), and repeated the Mann–Whitney *U* test with diversities not model-corrected for age.

Temporal Shifts in Diversification Rate. Temporal changes in diversification rate were visualized using a semilog lineage-through-time (LTT) plot. We tested the hypothesis of rate shifts occurring at major climatic events (EOGM and LOWE) by using two likelihood-based methods that use branching time information to compare models with a constant diversification rate to those with one or more rate shifts. The first method (36) treats branching times as analogous to failure times in statistical survival analysis. We used the second method (37) to compare a single-rate pure birth model with a three-rate model (with two shift points). These analyses assume complete lineage sampling within the period of interest (40 Ma to 19 Ma), which we believe our study approximates. However, because some excluded taxa may belong to unidentified, early-branching lineages, we repeated the second test using simulated incomplete phylogenies to gauge the potential effects of taxon sampling on our conclusions.

ACKNOWLEDGMENTS. We thank C. Labandeira, S. Wing, and C. Delwiche for helpful discussions and comments on early drafts. C. Labandeira brought to our attention the key leaf mine fossils, E. Righter and N. Reading provided valuable mathematical assistance, and two anonymous reviewers provided exceptionally helpful advice. This work was funded by the University of Maryland Graduate School and the National Science Foundation Grant 0531769.

1. Vrba ES (1985) Environment and evolution: Alternative causes of the temporal distribution of evolutionary events. *S Afr J Sci* 81:229–236.
2. Benton MJ (2009) The red queen and the court jester: Species diversity and the role of biotic and abiotic factors through time. *Science* 323:728–732.
3. Simpson GG (1953) *The Major Features of Evolution* (Columbia Univ Press, New York).
4. Jablonski D (2008) Biotic interactions and macroevolution: Extensions and mismatches across scales and levels. *Evolution* 62:715–739.
5. McKenna DD, Farrell BD (2006) Tropical forests are both evolutionary cradles and museums of leaf beetle diversity. *Proc Natl Acad Sci USA* 103:10947–10951.
6. Ehrlich PR, Raven PH (1964) Butterflies and plants: A study in coevolution. *Evolution* 18:586–608.
7. Winkler IS, Mitter C (2008) in *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects*, ed Tilmon KJ (Univ California Press, Berkeley), pp 240–263.
8. Berenbaum MR, Feeny PP (2008) in *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects*, ed Tilmon KJ (Univ California Press, Berkeley), pp 3–19.
9. Mayhew PJ (2007) Why are there so many insect species? Perspectives from fossils and phylogenies. *Biol Rev* 82:425–454.
10. Wheat CW, et al. (2007) The genetic basis of a plant-insect coevolutionary key innovation. *Proc Natl Acad Sci USA* 104:20427–20431.
11. McLeish MJ, Chapman TW, Schwarz MP (2007) Host-driven diversification of gall-inducing *Acacia* thrips and the aridification of Australia. *BMC Biol* 5:3.
12. Farrell BD (1998) Inordinate fondness explained: Why are there so many beetles? *Science* 281:555–559.
13. Marvaldi AE, Sequeira AS, O'Brien CW, Farrell BD (2002) Molecular and morphological phylogenetics of weevils (Coleoptera, Curculionoidea): Do niche shifts accompany diversification? *Syst Biol* 51:761–785.
14. Wiegmann BM, et al. (2000) Nuclear genes resolve Mesozoic-aged divergences in the insect order Lepidoptera. *Mol Phylogen Evol* 15:242–259.
15. Winkler IS, Scheffer SJ, Mitter C (2009) Molecular phylogeny and systematics of leaf-mining flies (Diptera: Agromyzidae): Delimitation of *Phytomyza* Fallén sensu lato and included species groups, with new insights on morphological and host-use evolution. *Syst Entomol* 34:260–292.
16. Spencer KA (1990) *Host Specialization in the World Agromyzidae (Diptera)* (Kluwer, Dordrecht, The Netherlands).
17. Scheffer SJ, Winkler IS, Wiegmann BM (2007) Phylogenetic relationships within the leaf-mining flies (Diptera: Agromyzidae) inferred from sequence data from multiple genes. *Mol Phylogen Evol* 42:756–775.
18. Wilf P, Labandeira CC, Johnson KR, Ellis B (2006) Decoupled plant and insect diversity after the end-Cretaceous extinction. *Science* 113:1112–1115.
19. Spencer KA (1973) *Agromyzidae (Diptera) of Economic Importance*, Series Entomologica (Junk, The Hague), Vol. 9.
20. Parella MP (1987) Biology of *Liriomyza. Annu Rev Entomol* 32:201–224.
21. Bremer B, et al. (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APGII. *Bot J Linn Soc* 141:399–436.
22. Jensen U (1995) Secondary compounds in the Ranunculiflorae. *Plant Syst Evol* (Suppl 9):285–297.
23. Seigler DS (1998) *Plant Secondary Metabolism* (Springer, New York).
24. Bremer K, Friis EM, Bremer B (2004) Molecular phylogenetic dating of asterid flowering plants shows early Cretaceous diversification. *Syst Biol* 53:496–505.
25. Moore BR, Donoghue MJ (2007) Correlates of diversification in the plant clade Dipsacales: Geographic movement and evolutionary innovations. *Am Nat* 170:S28–S55.
26. Behrensmeyer AK, et al., eds (1992) *Terrestrial Ecosystems Through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals* (Univ Chicago Press, Chicago).
27. Wolfe JA (1994) Tertiary climate changes at middle latitudes of western North America. *Palaeogeogr Paleoclimatol Palaeoecol* 108:195–205.
28. Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292:686–693.
29. Katz ME, et al. (2008) Stepwise transition from the Eocene greenhouse to the Oligocene icehouse. *Nat Geosc* 1:329–334.
30. Holbourn A, Kuhnt W, Schulz M, Erlendeuser H (2005) Impacts of orbital forcing and atmostpheric carbon dioxide on Miocene ice-sheet expansion. *Nature* 438:483–487.
31. Wolfe JA (1992) in *Eocene-Oligocene Climatic and Biotic Evolution*, ed Prothero DR, Berggren WA (Princeton Univ Press, Princeton), pp 421–436.
32. Graham A (1999) *Late Cretaceous and Cenozoic History of North American Vegetation North of Mexico* (Oxford Univ Press, Oxford).
33. Niklas KJ, Tiffney BH, Knoll AH (1985) in *Phanerozoic Diversity Patterns: Profiles in Macroevolution*, ed Valentine JW (Princeton Univ Press, Princeton), pp 97–128.
34. Mitchell A, Mitter C, Regier J (2006) Systematics and evolution of the cutworm moths (Lepidoptera: Noctuidae): Evidence from two protein-coding nuclear genes. *Syst Entomol* 31:21–46.
35. Magallón S, Sanderson MJ (2001) Absolute diversification rates in angiosperm clades. *Evolution* 55:1762–1780.
36. Paradis E (1997) Assessing temporal variations in diversification rates from phylogenies: Estimation and hypothesis testing. *Proc R Soc Lond B* 264:1141–1147.
37. Rabosky DL (2006) Likelihood methods for detecting temporal shifts in diversification rates. *Evolution* 60:1152–1164.
38. Ricklefs RE (2007) Estimating diversification rates from phylogenetic information. *Trends Ecol Evol* 22:601–610.
39. Rabosky DL (2009) Ecological limits on clade diversification in higher taxa. *Am Nat* 173:662–674.
40. Vieites DR, Min M-S, Wake DB (2007) Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proc Natl Acad Sci USA* 104:19903–19907.
41. Peña C, Wahlberg N (2008) Prehistorical climate change increased diversification of a group of butterflies. *Biol Lett* 4:274–278.
42. Tamura M (1993) in *The Families and Genera of Vascular Plants*, eds Kubitzki K, Rohwer JG, Bittrich V (Springer, Berlin), Vol 2, pp 563–583.
43. Graham A (1996) in *Compositae: Systematics. Proceedings of the International Compositae Conference, Kew, 1994*, eds Hind DJN, Beentje HJ (Royal Botanic Gardens, Kew), Vol 1, pp 123–140.
44. Zwickl DJ (2006) GARLI: Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD dissertation (Univ of Texas, Austin), available at http://www.nescent.org/wg_garli.
45. Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Mol Biol Evol* 19:101–109.
46. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214.
47. Maddison WP, Maddison DR (2005) Mesquite: A modular system for evolutionary analysis, Version 2.0, available at http://mesquiteproject.org.
48. Schluter D, Price T, Mooers A, Ludwig D (1997) Likelihood of ancestor states in adaptive radiation. *Evolution* 51:1699–1711.
49. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
50. Vamosi SM, Vamosi JC (2005) Endless tests: Guidelines for analysing non-nested sister-group comparisons. *Evol Ecol Res* 7:567–579.